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INTERACTION OF SCALABILITY PROCESS WITH ENVIRONMENTAL LIMITATIONS ON MICROALGAE CULTIVATION: LIGHT COLOR AND SALINITY EFFECTS

Interaksi Proses Skalabilitas dengan Batasan Lingkungan pada Kultivasi Mikroalga: Pengaruh Warna Cahaya dan Tingkat Salinitas

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ABSTRACT

Scaling up the cultivation of Chlorella vulgaris often results in differences in growth performance compared to laboratory-scale cultivation due to increased sensitivity to environmental factors. This study examined the interaction between light color, salinity, and the scale-up process at cultivation volumes of 1, 5, and 10 L using synthetic NaCl media and artificial seawater (ASW). Growth was monitored via optical density measurements. The results indicated that inoculum is a key factor factor influencing the success of scale-up. Cultures transferred during the logarithmic growth phase adapted more quickly and maintained exponential growth, while inoculum from the decline phase showed slower recovery and lower cell density. Red light minimized light stress and promoted higher cell density in both media. Lower salinity promoted faster growth and earlier peak attainment. Changes in dry biomass pigments indicate carotenoid accumulation under high salinity and light stress. The highest biomass yield was achieved when harvested during the logarithmic phase, with ASW medium providing higher productivity. Overall, optimizing inoculum age, light, and salinity is crucial for improving production efficiency during the C. vulgaris scale-up process.

Keywords: *Cultivation, C.vulgaris, Light color, Salinity, Scale-up*

ABSTRAK

Peningkatan skala budidaya Chlorella vulgaris sering menyebabkan perbedaan kinerja pertumbuhan dibandingkan skala laboratorium akibat meningkatnya sensitivitas terhadap faktor lingkungan. Studi ini mengkaji interaksi antara warna cahaya, salinitas, dan proses scale-up pada volume budidaya 1, 5, dan 10 L menggunakan media NaCl sintetis dan air laut buatan (artificial seawater, ASW). Pertumbuhan dipantau melalui pengukuran densitas optik. Hasil penelitian menunjukkan bahwa usia inokulum merupakan faktor kunci dalam keberhasilan scale-up. Kultur yang dipindahkan pada fase pertumbuhan logaritmik beradaptasi lebih cepat dan mempertahankan pertumbuhan eksponensial, sedangkan inokulum dari fase penurunan menunjukkan pemulihan yang lebih lambat dan kepadatan sel yang lebih rendah. Cahaya merah meminimalkan stres cahaya dan meningkatkan kepadatan sel yang lebih tinggi pada kedua media. Salinitas yang lebih rendah mempromosikan pertumbuhan yang lebih cepat dan pencapaian puncak yang lebih cepat. Perubahan pigmen biomassa kering menunjukkan akumulasi karotenoid di bawah stres salinitas dan cahaya yang tinggi. Hasil biomassa tertinggi dicapai saat panen pada fase logaritmik, dengan media ASW memberikan produktivitas yang lebih tinggi. Secara keseluruhan, optimasi usia inokulum, cahaya, dan salinitas sangat penting untuk meningkatkan efisiensi produksi selama proses scale-up C. vulgaris.

Kata kunci: *Kultivasi, C.vulgaris, Warna cahaya, Salinitas, Scale-up*

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INTRODUCTION

Chlorella vulgaris (*C. vulgaris*) is a photosynthetic unicellular microalga that is widely used in various fields, such as biotechnology, food, bioenergy, and the production of high-value compounds. It is also known for its high productivity – growing 15 to 300 times faster than terrestrial plants (Santoso, 2016) – high adaptability, and lipid content of 14-22% of its dry weight (Irhamni, et al., 2014). Additionally, *C. vulgaris* produces a variety of high-value bioactive chemical compounds (Safrida, et al., 2025).

To produce more biomass and its derivatives, the cultivation process needs to be scaled up to a larger volume. However, growing microalgae cultures on a large-scale often does not result in the same growth yields as those achieved in smaller (laboratory) cultures. The process of scaling up cultivation can induce changes in the physiological response of cells and increase the sensitivity of cultures to environmental factors. Ratomski and Hawrot-Paw (2021) state that optimal conditions enable maximum cell growth, while environmental limitations play a role in triggering lipid accumulation as its response to stressful conditions.

Masitoh et al. (2022) reported that excessive light intensity is detrimental to photosynthetic pigments, while lower intensity promotes biomass accumulation. In terms of cultivation, salinity is another factor that poses a challenge for microalgae. Ion imbalance and osmotic stress, as well as the presence of reactive oxygen species (ROS) due to saline conditions, can disrupt the photosynthetic process of microalgae (Shetty, et al., 2019). *Chlorella* requires chloride ions for ATP and flavin mononucleotide (FMN) biosynthesis during photosynthesis. At a concentration of 0.5 M sodium chloride (NaCl), lipid content increased to 21.4%, but biomass decreased (Rai et al. 2015).

On a laboratory scale, salinity changes can be controlled, and any fluctuations can be corrected immediately. But in larger-scale cultivation systems, this kind of control becomes much more demanding, and even small fluctuations can have a significant impact on culture stability. These conditions indicate that the interaction between the scale-up process and environmental limitations determines the success of biomass and metabolite production of *C. vulgaris*. In order to understand the dynamics of change that occur during scale-up process, experimental studies are needed to evaluate the response of cultures against limited environmental factors. Based on this, this study focuses on analyzing the growth of *C. vulgaris* in saline media through two scale-up stages, while also evaluating the effects of environmental factors, e.g., light intensity and salinity levels, on the scale-up process. This knowledge allows for a deep understanding of the cell culture response, thereby reducing the risk of mass production failure can be suppressed at volumes higher than 10 litres.

MATERIALS AND METHODS

Materials

The main material used in this study was *C. vulgaris* sourced from marine habitats and obtained commercially from an external laboratory in Klaten Regency. The synthetic medium was prepared using commercially available salts enriched with F/2 Guillard (laboratory standard), with volume ratio of 1:0.002 (v/v). The salt concentrations used were 0.5, 0.25, and 0 M (without salinity). In addition, commercial sodium hydroxide (NaOH) solids were used as a material for the harvesting process.

Place and Time of Research

This research was conducted experimentally from February to November 2025 at the Chemical Engineering Laboratory, Institut Teknologi Sumatera, South Lampung, Lampung, Indonesia.

Cultivation Process

Initial cultivation was carried out in 3 bioreactors, coded A1, A2, and A4, each with a total volume of 1 liter. *C. vulgaris* inoculum was added at 10% of the total volume to a medium with a salinity of 0.5 M. During the cultivation process, aeration was carried out continuously. During cultivation, cell density (OD) measurements were performed using a UV-Vis spectrophotometer (A&E LAB, UV-1000) at a wavelength of 685 nm. OD measurements were conducted

daily in the morning and afternoon, with two samples taken at each time, except on Saturdays and Sundays, when no measurements were taken. After measuring the OD, 0.2 mL of F/2 Guillard solution was added to the 1-liter culture. The lighting period lasts 24 hours with white light. The following cultivation stage was carried out by progressively scaling up to a volume of 5 liters (Scale-up with light intensity limitations) and then 10 liters (Scale-up with salinity level limitations), without replication.

a. Scale up with light intensity limitations

Subsequent cultivation was performed in a total volume of 5 liters. A 10% of *C. vulgaris* inoculum from a 1-liter culture was transferred into fresh medium with a salinity of 0.5 M. Continuous aeration was provided throughout the cultivation process. Cell density (OD) measurements were performed as previously described, following which 1 mL of F/2 Guillard solution was added to the 5-liter culture. The 5-liter culture was maintained under a 24-hour light cycle using white light (code: **P5L**) and red light (code: **M5L**). Light intensity measurement by digital luxmeter (KUBER-AS803).

b. Scale-up with salinity level limitations

Further cultivation was carried out on a total volume of 10 liters. A 10% of *C. vulgaris* inoculum from the M5L culture was taken and added to fresh medium with varying salinities of 0.5 M (code: **M10La**), 0.25 M (code: **M10Lb**), and without salinity (code: **M10Lc)**. Continuous aeration was maintained during the entire cultivation process. Cell density (OD) was measured as previously described after which 2 mL of F/2 Guillard solution was added to the 10-liter culture. Photo period was 24 hours with red light.

Biomass harvesting and drying

Biomass harvesting is carried out through a chemical flocculation using 10% NaOH solution. The ratio of flocculant to culture is 0.2:1 (v/v). The flocs formed are then separated through a filtration process. Before harvesting, the final pH of the culture is measured using a pH meter (KEDIDA CT-6020A). The wet biomass obtained is then dried at 80 °C in an oven (MEMMERT 30 L).

Interpretation data

The OD data used in the interperation are the average daily OD values. The dry weight of the biomass is employed to figure out the biomass yield per liter of culture.

RESULTS AND DISCUSSION

Initial cultivation of microalgae in three bioreactors using white light and NaCl synthetic medium, each with a capacity of 1 liter, coded A1, A2, and A4. Initial cultivation was carried out under the same conditions in the three containers. The absorbance curves for the growth of cultures A1 and A2 are shown in **Figure 1**.

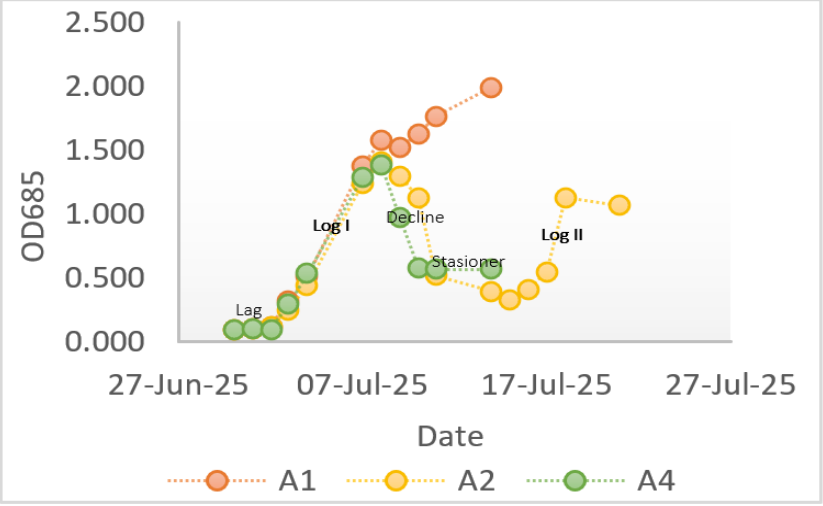


Figure 1 Growth profile of C. vulgaris in a 1-liter

The cultivation period for A1 and A4 is 14 days, while A2 is extended to 21 days. The initial absorbance values for A1, A2, and A4 are 0.088, 0.089, and 0.090, respectively. After 2 days of cultivation adaptation, distinctions began to emerge, with A1 exhibiting higher absorbance values than A2 and A4 until day 8. Although the initial inoculum volume was the same, differences in OD may have occurred because microalgae cells in each culture responded differently to growth factors (Shuler and Kargi, 2002). Optical density is one of the simplest and most practical methods for measuring cell density in culture. A higher OD indicates that more light is being absorbed by the microalgae in the culture, which is indicated by a dense green color in the culture. However, this method is quite challenging when it comes to relating optical density values to the actual number of cells (Beal, et al., 2020).

Next, culture A2 shows a decrease in OD and lasts for 7 days, then returns to rapid growth for 3 days, indicating a secondary logarithmic phase, before finally falling again. The first logarithmic phase (Log I) in A2 lasts longer, for 6 days, with a higher OD value compared to the secondary logarithmic phase (Log II), which lasts only 3 days. The peak growth in the log II phase was lower due to the filtering process carried out on day 11 in culture A2, which aimed to separate dead biomass from living biomass before cultivation continued. There is a possibility, though that some living cells which had increased in size were also trapped during the filtering process, causing a decrease in cell density. After 21 days, culture A2 was used as inoculum for 5 L cultivation with white light illumination, code P5L.

Meanwhile, the A4 culture was divided into two equally sized portions, each placed in a 2 L container filled with fresh media with a salinity of 0.5 M. The two containers were labeled P2La (using synthetic NaCl) and P2Lb (using ASW/artificial seawater). ASW salt is a synthetic mixture formulated to resemble the composition of seawater and is used as a medium for culturing marine microorganisms. The use of ASW salt makes it possible to create a more consistent marine environment than using natural seawater, as it provides the macronutrients needed by marine biota but is free from parasites and bacteria. In addition, *C. vulgaris* is also considered to have the ability to desalinate the salt content in the cultivation medium. This biodesalination process occurs through a metabolic mechanism that is independent and reversible, involving bioadsorption and bioaccumulation (Hasan, et al., 2023). The cultivation medium in this study was also enriched with F/2 Guillard as an additional nutrient source, which contains inorganic salts and other important compounds that support microalgae growth (Widyatama, et al., 2024). In this study, the salinity of the 0.25 and 0.5 M salt media was measured by Salinometer with results of 14.8 and 20.9 ppt, respectively.

The P2La culture was cultivated for 33 days, while the P2Lb culture was cultivated for 43 days (**Figure 2**). Based on the absorbance value as an indicator of microalgae cell concentration, it can be seen that P2Lb showed a more significant increase in growth compared to P2La. At the beginning of cultivation, cells in the ASW salt-based medium, P2Lb, undergo an active growth phase for 3 days, then experience a decline in cell count for the next 3 days, before re-entering the active growth phase. In contrast, the P2La culture only undergoes a short active growth phase, for 5 days (July 23-28, 2025), then enters a stationary phase for the 18 following days. On the contrary, after the 11th day, A1 was used as inoculum in a 5 L cultivation container with red light. This culture was distributed 500 mL each into two containers containing fresh media (0.5 M synthetic NaCl), which were then coded M5L, it was done in duplicate (code: M5La and M5Lb). The initial OD for M5La and M5Lb were 0.281 and 0.291, respectively.

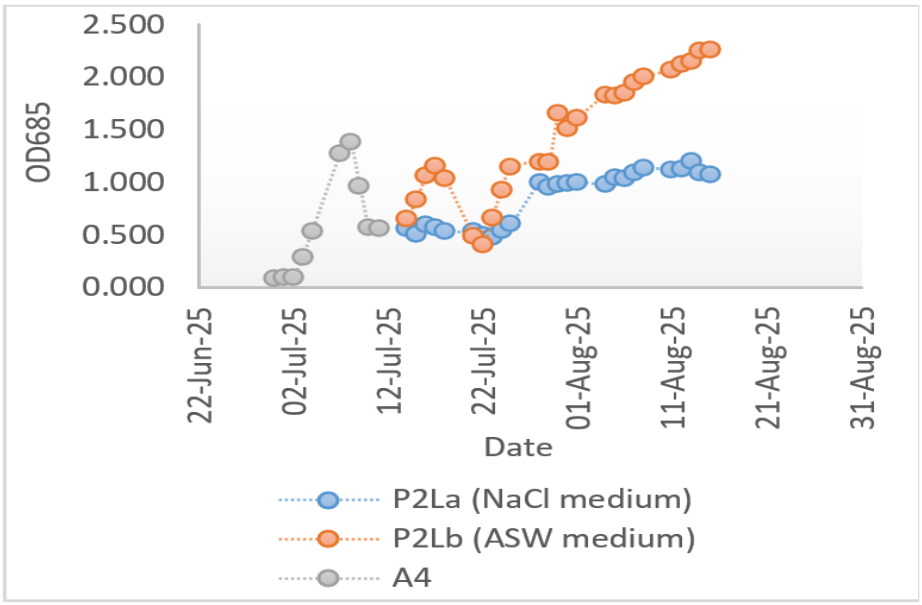


Figure 2 Growth curve of *C. vulgaris* over 47 days from a cultivation scale of 1 L (code: A4) to 2 L (code: P2L)

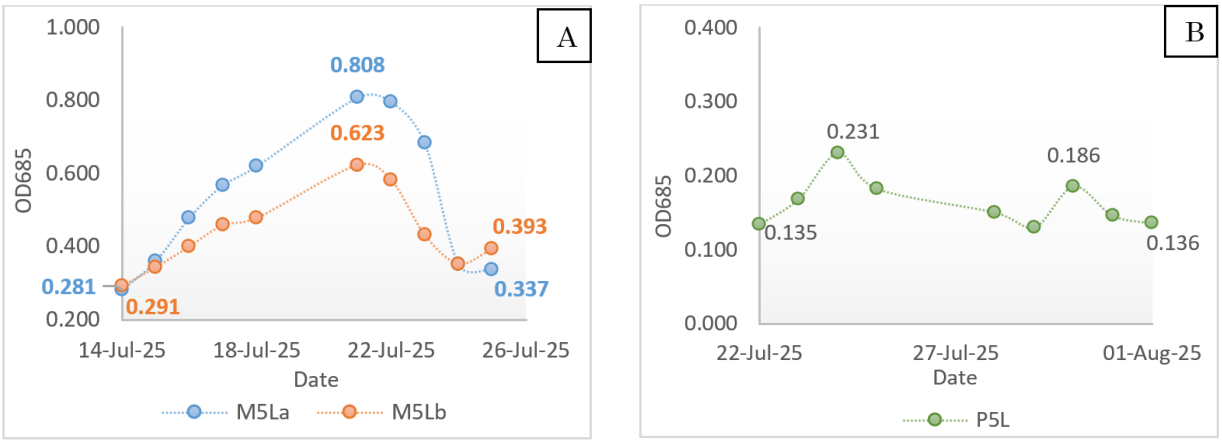


Figure 3 (A). Comparison of *C. vulgaris* growth in M5La and M5Lb cultures, (B). Growth pattern of *C. vulgaris* in P5L cultures over 11 days

Based on **Figure 3 (A),** the growth curves of M5La and M5Lb show a similar pattern, forming a dome, with the peak of the M5La curve higher than that of M5Lb. Its cultivation took place over 11 days and immediately entered a rapid growth phase, without the need for re-adaptation. The rapid growth phase peaked on the 7th day, after which the culture entered a decline or death phase. On July 25, the M5La culture was used as inoculum in a 10-liter container. After scaling up, fresh media was added to the remaining M5La culture and further growth (cultivation II) was carried out. Like the 11-day M5L culture, the P5L culture has an initial optical density of 0.135. This value is lower than that of M5L because the inoculum sources used are different, with P5L originating from the A2 culture and M5L originating from the A1 culture. Consistent with its initial conditions, the peak density of the P5L culture was also lower than that of the M5L culture during 11 days of cultivation.

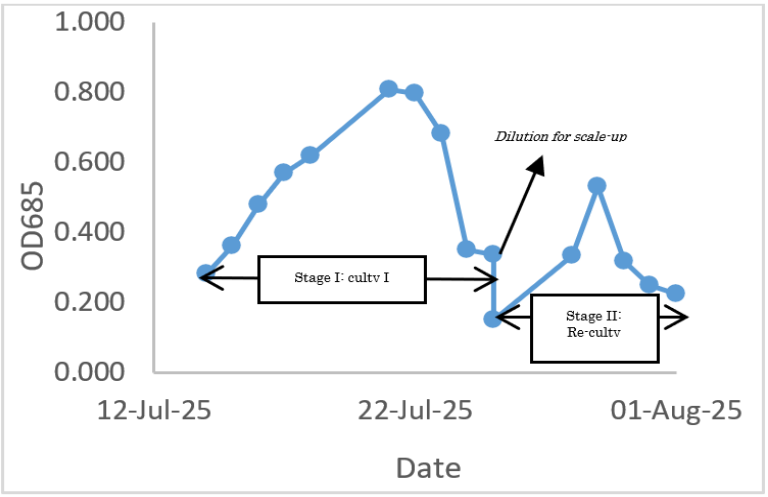


Figure 4 Growth profile of *C. vulgaris* in a 5-liter culture in NaCl medium with red light

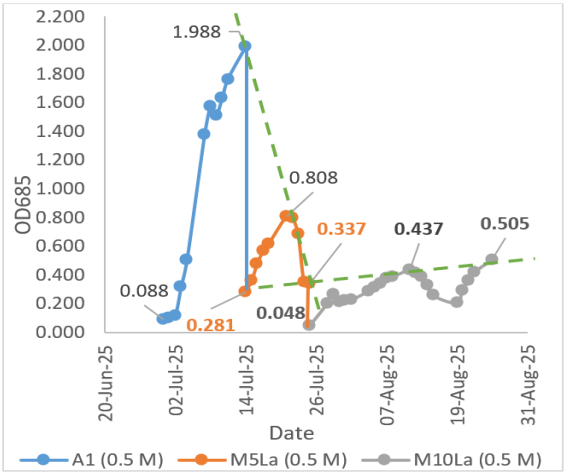
Figure 4 shows changes in the absorbance value of the M5La culture over time during the two stages of the cultivation process. In cultivation stage I, the OD increased rapidly between July 14 and 21, 2025. After achieving the maximum OD value, the first peak, of 0.808, the growth rate declined sharply, which may have been caused by the depletion of nutrients. After dilution on July 25, 2025, the M5La culture again showed an active growth phase until it reached a second peak with an OD value of 0.532 on July 29, 2025. The active growth time in cultivation II (re-cultivated) was shorter, only 4 days, while cultivation I lasted 7 days. The rapid growth surge in cultivation II (re-cultv) caused nutrients to be depleted immediately because they were not proportional to the number of cells growing, resulting in a shorter logarithmic phase. It should be noted that the M5La cultivation method is semi-continuous, which involves removing part of the culture volume and replacing it with the same amount of fresh media. The semi-continuous system has the advantage of maintaining cell growth, but this system limits lipid accumulation (Oyama, et al., 2022). During active growth (log phase), microalgae do not produce large amounts of lipids (Tan and Lee, 2016).

Variation of salinity level.

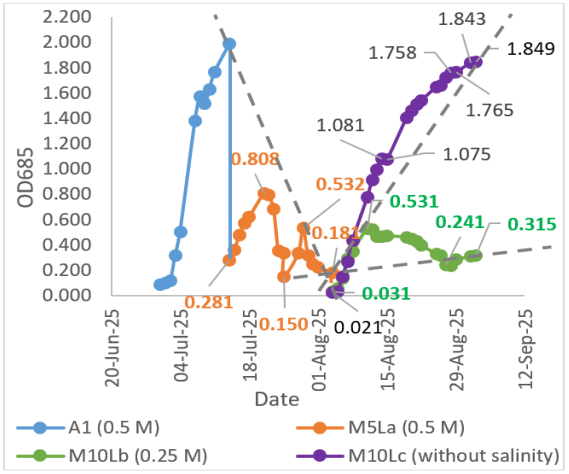
The inoculum for the M10La culture was taken from M5La in growth stage I (Cultv I) on July 25, 2025, while the inoculum for the M10Lb and M10Lc cultures was taken from M5La in growth stage II (re-cultv) on August 4, 2025. The initial absorbance values of each culture were 0.048 for M10La, 0.031 for M10Lb, and 0.021 for M10Lc (**Figure 5**). The three containers were cultivated for 29 days, then each container was immediately harvested. Each M10L culture showed a peak density at different times. In the 0.5 M culture (M10La), the culture accomplished two peaks, consecutively on the 17th day and the last day, the 29th day. Unlike M10La, which still showed a gradual increase in peak density, M10Lb showed a decline in peak growth. Meanwhile, the culture without salinity (M10Lc) showed a gradual increase in OD, with three times of deceleration phase without decreasing OD.

At the same salinity concentration, **Figure 5 (a),** the three cultures showed different growth responses, indicating that the success of the scale-up is not only determined by salinity but also by the physiological condition of the inoculum. Culture A1, which was used as the inoculum for the 5-liter culture (M5La), was in the log phase with the highest cell density. When A1 was transferred to M5La, there was a decrease in cell density due to dilution. However, the M5La culture showed a strong growth response as it immediately re-entered the log phase. Otherwise, the M10La culture experienced slower growth due to the use of M5La inoculum which was already in the decline phase. The trend of cell density change for inoculum A1 showed a sharp linear decrease from its peak point (OD = 1.988), then passed through the

M5La peak point (OD = 0.808) up to its endpoint (OD = 0.337). Meanwhile, the change in cell density for the M5La inoculum showed a more gradual increase to the first peak point (OD = 0.437) up to the M10La endpoint (OD = 0.505). The low M10La peak indicates that the culture was still alive but weak, as the cells were not able to actively proliferate again. This condition was also exacerbated by the use of an inoculum already in the decline (death) phase, leading to slow culture development because the number of dead cells was greater than the live ones.



(a) *Scale-up* culture at the same salinity



(b) *Scale-up* culture at different salinity

Figure 5 The effect of salinity differences on culture growth profiles (NaCl medium). The dotted line on the graph indicates the density changes of cells from the inoculum after scale-up.

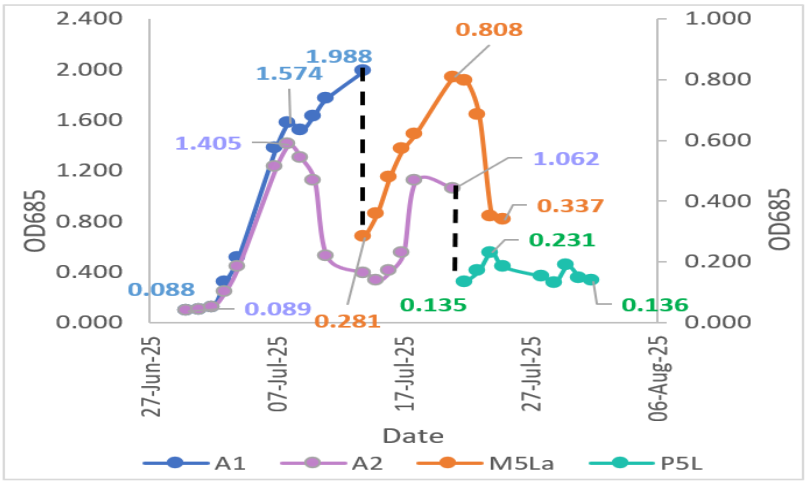


Figure 6 The effect of different light intensities on the growth profile of the culture (NaCl medium). The dotted line on the graph indicates a decrease in OD due to scale-up

When salinity was reduced, as shown in **Figure 5 (b),** the ability of the cells in the culture to resume growth changed noticeably. Although M10Lb exhibited slow growth, it experienced only a moderate level of stress compared to M10La, as indicated by its higher cell density and the faster attainment of its peak within 10 days. Meanwhile, the response of cell density changes from the M5La inoculum—after undergoing re-cultivation—to the M10Lb and M10Lc cultures displayed markedly different growth patterns. This is evident from the slope of the gray dashed line in **Figure 6 (b)**, where the steeper slope observed in M10Lb suggests a lower level of stress compared to M10Lc. These findings confirm a negative correlation between

salinity and growth performance. However, salinity does not act as the sole determining factor, inoculum density and physiological age also play crucial roles. Selecting an inoculum in the active growth phase (log phase) should be considered during scale-up to ensure a greater likelihood of achieving optimal culture development. This is further supported by research by Cheng et al. (2018) explained that inoculum age significantly influences rapid cell growth.

Variation of light intensity.

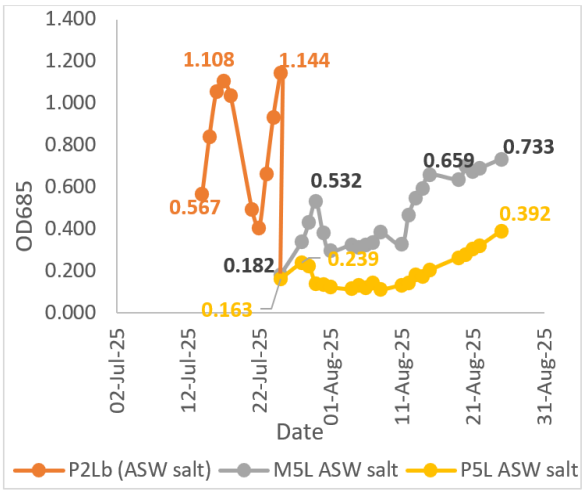
The A1 culture, initially grown under white light, reached the active growth phase before being scaled up to M5La. During the scale-up process, the cells were not only transferred to fresh media but also experienced a change in light from white to red. Hence the A1 inoculum is moved while it is still in the active growth phase, the cells can immediately continue the exponential phase again. Active growth in M5La is also influenced by a decrease in light intensity, considering that white light (~2700 lux measured) has a higher intensity than red light (~1500 lux measured), so the level of stress caused by light in cells is reduced (**Figure 6**). Even A2 had been in a decline phase for 6 days, although it was able to re-enter the active growth phase, but its peak OD was lower. During the scale-up process from A2 to P5L, no change in light intensity occurred, so the lighting conditions did not provide any additional benefit to support the recovery or further enhancement of the culture's growth.

Figure 7 Color of the dry biomass of *C. vulgaris* in culture: (a). M10Ld 0.5 M, (b). M10Le 0.25 M, (c). M10Lc (without salinity), (d). M5L ASW, and (e). P5L ASW

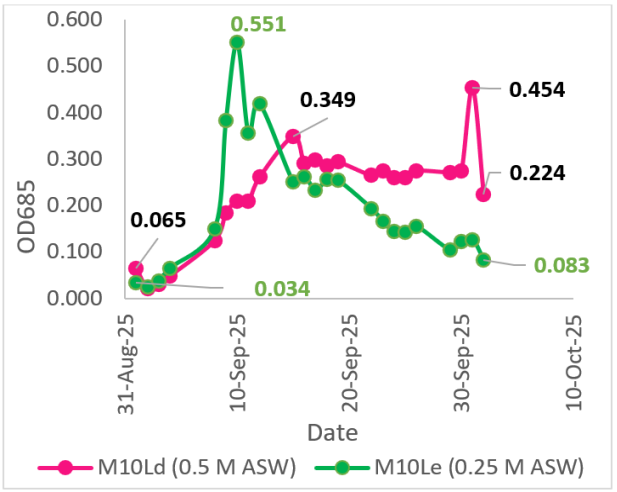
Light intensity and salinity levels during cultivation affects the color of its dry biomass. In **Figure 7 (c),** *C. vulgaris* cultivated without salt addition looks colored green, indicating the dominance of chlorophyll pigments. Contrary to this, the other four biomass samples show non-green colors, different with the typical green color of *C. vulgaris*. This color change indicated a pigment shift from chlorophyll to complementary pigments, such as carotenoids. Excessively high light intensity has the potential to damage the function of chlorophyll pigments, but the presence of carotenoids acts as a protector by absorbing excess light energy during photosynthesis [Febrieni, et al., 2020]. Carotenoid pigments create colors in the red, orange, and yellow range, but are prone to fading due to oxidation [Wahyuni, et al., 2020]. Thus, variations in dry biomass color reflect the response of pigments to environmental conditions during growth. High salinity creates osmotic pressure that inhibits nutrient absorption [Nisa, et al., 2020], causing cell growth to become unstable. However, cells that are able to survive under osmotic stress tend to increase carotenoid synthesis. The increase in pigment accumulation was confirmed visually, where

the dry biomass showed a pink color, for the highest salinity culture (0.5 M). The color of carotenoids comes from their central chromophore, which is a conjugated double bond that acts as a light absorber. Seven conjugated double bonds produce a pale yellow color. The more conjugated double bonds in the carotenoid, the deeper the color, shifting from yellow to orange and even red. Additionally, factors such as pigment concentration and aggregation, as well as carotenoprotein formation (interaction with proteins), also impact color, even expanding the color range to brown [Rodriguez-Amaya, et al., 2023]. In this study, the number of lamps and attached wattage are identical, but it produce different light intensity because different colored lamps emit different spectrums.

The P2Lb culture based on ASW media also went through a scale-up process, with its growth pattern shown in **Figure 8**, indicating similar growth limitations to cultures on synthetic NaCl media. Both the 5-liter and 10-liter cultures were cultivated for 31 days before harvesting. Light intensity limitation produced the same trend, i.e., red lighting produced higher OD values than white lighting. Meanwhile, the effect of salinity level limitation cannot be compared because the source of inoculum is different in synthetic NaCl media.



(a) Effect of light intensity



(b) Effect of intensity level

Figure 8. Growth profile of C. vulgaris on ASW medium

The peak density of the M10Le culture was reached faster, on the 10th day, whereas the M10Ld culture on the 15th day. After reaching this peak, neither the M10Ld nor the M10Le culture showed any further log growth until the end of cultivation. Although M10Ld reached its peak later than M10Le, its density at the end of cultivation was higher than M10Le. Physiologically, this can be attributed to the metabolic response of cells to osmotic pressure. Exposure to higher salinity has the potential to cause natural selection, in which weak cells will be eliminated. The remaining cells are usually more metabolically resistant, although they no longer show a continued log growth phase.

Metabolic changes are the microalgae's response to osmotic stress [Esteves, et al., 2025]. To balance these conditions, cells usually accumulate osmoprotective molecules, such as proline, which act as osmolytes [Fal, et al., 2022]. In contrast, M10Le, which reached its peak faster, showed good initial performance but was unable to maintain high cell density until the end of the cultivation period. It turns out that the point of scaling up is not merely to boost the number of cells, but to understand the biological resilience of each cell and how its respond to changes in environmental conditions.

Table 1. Biomass produced for each culture

	End phase of the culture harvest	Yield biomassa (gr dry weight/L)
M5Lb (NaCl medium)	Stationer	2.520
P5L (NaCl medium)	Stationer	2.040
M5L (ASW medium)	Deseleration	3.782
P5L (ASW medium)	Logarithmic	4.396
M10La	Stationer	0.245
M10Lb	Stationer	0.232
M10Lc	Logarithmic	4.188
M10Ld	Stationer	3.307
M10Le	Stationer	1.979

Regarding harvesting (**Table 1**), the biomass yield of *C. vulgaris* obtained varies. Harvesting during the logarithmic phase produces the highest biomass yield, followed by the deceleration phase and the stationary phase. For media used, ASW salt provides better growth compared to synthetic NaCl salt.

CONCLUSION

This study demonstrates that the success of *C. vulgaris* cultivation during scale-up is strongly influenced by environmental limitations, particularly light intensity and salinity level, as well as the physiological condition of the inoculum. Cultures initiated from inocula in the active logarithmic phase consistently showed faster recovery and higher growth rates after scale-up compared to inocula taken during the decline phase. Red light illumination, which provides lower light intensity than white light, reduced photostress and supported higher optical density in both NaCl-based and ASW-based media. Salinity variation produced distinct growth patterns, confirming a negative correlation between salinity and culture performance; lower salinity enabled faster peak attainment and reduced stress levels. Nevertheless, salinity was not the sole determining factor, as inoculum age and density significantly affected culture response. ASW-based media supported better biomass accumulation than synthetic NaCl, indicating improved nutrient balance and stability. Biomass harvested during the logarithmic phase yielded the highest dry weight, followed by deceleration and stationary phases. Overall, the interaction between scale-up procedures and environmental constraints dictates culture stability and productivity. Optimizing inoculum physiological age, light intensity, and salinity conditions is therefore essential to achieving efficient biomass production during large-scale cultivation of *C. vulgaris*.

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